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ANTIBODIES TO MARINE CALICIVIRUSES IN THE PACIFIC WALRUS (*ODOBENUS ROSMARUS DIVERGENS* ILLIGER)[1]

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ABSTRACT: Sera from 155 Pacific walruses (*Odobenus rosmarus divergens* Illiger), sampled in the Chukchi Sea during the summer of 1983, were tested for serum neutralizing (SN) antibodies to six marine calicivirus serotypes. Serotypes tested included San Miguel sea lion virus (SMSV) types 1, 5, 8, and 10, previously isolated from northern fur seals (*Callorhinus ursinus* Linné) in the Bering Sea; walrus calicivirus (WCV), previously isolated from walrus feces collected off sea ice in the Chukchi Sea; and Tillamook calicivirus (TCV), a bovine isolate from Oregon of suspected marine origin. No antibodies were found to SMSV-1, SMSV-10, or TCV. Antibodies to SMSV-5 were found in two animals (titers 1:20 and 1:160); antibodies to SMSV-8 were found in four animals (all 1:20); and antibodies to WCV were found in one animal (titer 1:40). Antibodies to WCV have been found in the Pacific walrus previously; however, this represents the first report of antibodies to any of the SMSV serotypes in this marine mammal.

INTRODUCTION

Previous reports indicate that caliciviruses are present in numerous marine species within the Pacific Ocean basin (Smith, 1981; Barlough et al., 1986). Since 1972 11 distinct caliciviruses, the San Miguel sea lion viruses (SMSV's), have been isolated from marine mammals and from an ocean fish (opaleye, *Girella nigricans* Ayres). The California sea lion (*Zalophus c. californianus* Lesson) and the opaleye are believed to be intimately involved in maintenance of the SMSV epizootic/enzootic cycle in coastal waters of southern California (Smith et al., 1976, 1980; Smith, 1981; Barlough et al., 1986). Cycles of calicivirus in far northern waters, however, remain poorly characterized. It has been proposed that certain migratory species, most notably the northern fur seal and the California gray whale (*Eschrichtius robustus* Lilljeborg), may be infected by SMSV's in southern California waters and subsequently carry the agents northward to the Bering Sea (Smith et al., 1980). In fur seals, SMSV's appear to pass through the northern herds in epizootic form and then disappear, suggesting that appropriate reservoirs for over-wintering or long-term maintenance of caliciviruses are not present (Smith et al., 1976).

In 1977 a distinct calicivirus serotype, the walrus calicivirus (WCV), was isolated from Pacific walrus (*Odobenus rosmarus divergens* Illiger) feces collected off sea ice in the south-central Chukchi Sea (Smith et al., 1983b). To date there is no evidence, either virological or serological, linking this agent to any other animal species. This, combined with the recent finding (Smith et al., 1981a) of calcivirus antibodies in bowhead whales (*Balaena mysticetus* Linné)—another species which, like the walrus, is confined year-round to the shifting margins of the Arctic pack ice—suggests that northern reservoirs capable of maintaining calicivirus cycles may exist.

Here we present the results of a serologic investigation intended to estimate, for the first time, the degree of exposure of the Pacific walrus to a number of marine calicivirus serotypes. The ultimate aim

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TABLE 1. Antibodies to marine caliciviruses in sera from Pacific walruses (*Zykovo* cruise, 1983).

Neutralizing antibody titers (100% endpoint)	Virus					
	SMSV-1	SMSV-5	SMSV-8	SMSV-10	Walrus CV*	Tillamook CV
<1:20	155	153	151	155	154	155
1:20	0	1	4	0	0	0
1:40	0	0	0	0	1	0
1:80	0	0	0	0	0	0
1:160	0	1	0	0	0	0
Total positive/no. tested (% positive)	0/155	2/155 (1.3%)	4/155 (2.6%)	0/155	1/155 (0.65%)	0/155

* CV = calicivirus.

of our work, to which this report is preparatory, is to determine the role of the Pacific walrus (and other northern species of marine mammals) in the maintenance of calicivirus cycles in far northern waters.

MATERIALS AND METHODS

Sera were collected in July and August 1983 from 155 walruses, of mixed age and sex, harvested aboard the commercial Soviet sealing vessel *ZRS Zykovo* (Lowry and Fay, 1984). The cruise track covered extensive near-shore (Chukotka) and ice-front leads, generally southeast of Wrangell Island, in the Chukchi Sea. Sera were tested for serum neutralizing (SN) antibodies to the five recognized calicivirus serotypes previously isolated from northern marine mammals (SMSV-1, SMSV-5, SMSV-8, SMSV-10, and WCV) (Barlough et al., 1986), and for antibodies to Tillamook calicivirus (TCV), a recent bovine isolate from Oregon (Smith et al., 1983a) suspected to be of marine origin. A microtiter (96-well) SN procedure using Vero cells was performed, first to screen sera at a dilution of 1:20, and then to titrate positive samples (Smith et al., 1976). Serum-virus mixtures were incubated for 60 min at room temperature prior to addition of cells. The antibody titer was defined as the highest dilution of serum completely neutralizing 100 $TCID_{50}$ of virus in all four replicate test wells (100% end-point). Tests for the SMSV's and for TCV were read after 72 hours' incubation. The WCV required 5 days' incubation because of its slower, more cell-associated cytopathology. Type specificities were checked by neutralization and reciprocal cross-neutralization with the appropriate rabbit hyperimmune antisera (Smith et al., 1983a).

RESULTS AND DISCUSSION

Seven of 155 (4.5%) sera contained calicivirus-neutralizing activity (Table 1). Antibodies were found to SMSV-5, SMSV-8, and WCV. As shown in Table 2, all seven positive results were obtained from separate individuals. Most notable were the 1:40 titer to WCV in walrus 085 and the 1:160 titer to SMSV-5 in walrus 067. All calicivirus serotypes were neutralized only by their respective rabbit hyperimmune antisera in neutralization and reciprocal cross-neutralization tests.

Since the original isolation of WCV, antibodies to this agent have been found in several Pacific walruses (Smith et al., 1983b). Our additional finding of antibodies to SMSV-5 and SMSV-8 in walruses represents the first evidence of exposure of this marine mammal species to any of the SMSV's. However, the low prevalence of antibodies to all three viruses (Table 1) suggests that none was actively epizootic during the summer of 1983 in the walrus population sampled (Smith et al., 1976). This was not totally unexpected, considering that these agents were isolated originally several years before the *Zykovo* cruise (SMSV-5 in 1973, SMSV-8 in 1976, and WCV in 1977) (Barlough et al., 1986). Nearly thirty distinct marine calicivirus serotypes have been recovered from a number of animal species over the past 50 yr; in no case has any serotype been reisolated more than 3 yr after its original recovery (Barlough et al., 1986). Whether this sequential isolation of viruses represents continual emergence of new serotypes through natural genetic change, or

TABLE 2. Neutralizing antibody profiles of seropositive Pacific walruses.

Animal no.	Sex	Age (yr)[a]	Virus					
			SMSV-1	SMSV-5	SMSV-8	SMSV-10	Walrus CV[b]	Tillamook CV
042	F	22	Neg[c]	Neg	1:20	Neg	Neg	Neg
067	F	14	Neg	1:160	Neg	Neg	Neg	Neg
082	F	13	Neg	1:20	Neg	Neg	Neg	Neg
085	M	14	Neg	Neg	Neg	Neg	1:40	Neg
212	F	16	Neg	Neg	1:20	Neg	Neg	Neg
284	F	29	Neg	Neg	1:20	Neg	Neg	Neg
291	M	14	Neg	Neg	1:20	Neg	Neg	Neg

[a] Determined by dental examination (Fay, 1982).
[b] CV = calicivirus.
[c] Negative result = <1:20.

simply random selection from a large pool of circulating serotypes, has been the subject of unresolved discussion for many years (Smith, 1981; Barlough et al., 1986).

The primary route of transmission of marine caliciviruses is believed to be in the food chain (Smith et al., 1976, 1980, 1983a, b; Smith, 1981; Barlough et al., 1986). In southern California coastal waters, certain ocean fishes such as opaleye apparently serve as reservoirs of calicivirus for their natural predator, the California sea lion (Smith et al., 1980; Smith, 1981; Barlough et al., 1986). In Arctic and subarctic regions, several pinniped and cetacean species have been found to carry antibodies to caliciviruses, but a northern maintenance cycle for these agents has yet to be identified (Smith et al., 1976, 1981a, 1983b; Barlough et al., 1986). Because fishes comprise an insignificant portion of the diet of the Pacific walrus (Fay, 1982), it seems unlikely that they would provide a significant, direct source of virus for this species. However, the possibility still exists that fishes may serve as northern reservoirs, or perhaps as intermediate transport hosts, ferrying caliciviruses from one species to another within the walrus's habitat. The principal foods eaten by walruses are bivalve molluscs (clams, mussels) (Fay, 1982). These filter-feeders are efficient concentrators of water-borne microbial agents (e.g., enteroviruses) (Sobsey, 1982), and thus might be important in maintaining calicivirus infection cycles among walrus populations (and elsewhere). Prolonged calicivirus survival in saltwater has been documented (Smith et al., 1981b), but no caliciviruses have been isolated to date from molluscs. Alternatively, walruses are known to prey on other pinnipeds occasionally (Lowry and Fay, 1984), and thus might acquire calicivirus infections directly in this manner. Occasional or casual physical contact with other pinniped species—for example, northern fur seals in the Bering Sea—is considered an unlikely mode of interspecies transmission, as discussed previously (Smith et al., 1983b).

Although it is not possible at this time to assess the impact of caliciviruses on the health of the Pacific walrus population, these agents are known to cause vesicular lesions, probably abortion, and possibly encephalitis and pneumonitis, in other pinniped species (Smith et al., 1980; Smith, 1981; Barlough et al., 1986). However, recent observations of reproductive disturbances among Pacific walruses (Fay et al., 1984) may warrant investigation of possible calicivirus involvement in this species. It also must be kept in mind that the low prevalence of neutralizing antibodies in the sample population may not necessarily reflect lack of widespread exposure to these agents. In enzootic areas (e.g., south-

ern California waters), antibody titers to specific calicivirus serotypes typically are low and of sporadic occurrence, except during periods of epizootic activity (Smith et al., 1976; Berry et al., unpubl. data). Thus most of the antibody titers detected in walruses in this study probably reflect exposure during past epizootics. Elevated titers under these circumstances (e.g., walrus 067) may indicate rare animals that have experienced recent infection with enzootic serotypes.

Further work to isolate caliciviruses from molluscs and fishes resident within the walrus's habitat should be helpful in elucidating the natural history of these agents in far northern waters, and in the identification of northern reservoirs. The possibility exists, however, that walruses themselves may act as primary reservoir hosts, passing caliciviruses directly from one to another, or through filter-feeding intermediates such as bivalve molluscs.

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